

## COMPARISON OF ELISA AND RAPID SCREENING TESTS FOR THE DIAGNOSIS OF HIV IN HIGH RISK INDIVIDUALS

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### ABSTRACT

**Background:** Human Immunodeficiency Virus infection is the fourth leading cause of death world-wide and thus, remains a major public health and socio-economic concern globally. Testing for HIV is a very important component of HIV/AIDS prevention strategies due to the fact that an alarming number of the People living with HIV/AIDS (PLWHA) remain unaware of their infection, hence, spreading the infection in the community.

**Objectives:** This study was to determine significant difference, if any, in the prevalence of HIV infection among high risk group if tested with more than one method of screening for HIV infection.

**Materials and Methods:** Spouses of HIV reactive patients at Ladoke Akintola University Teaching Hospital were counselled and made to undergo voluntary testing for HIV. This was done by testing for HIV p24 core antigen (ELISA) in their blood, and also with the rapid screening for HIV antibodies with the parallel use of Determine strips plus Uni gold kits and Stat pack as a tie breaker. Data analysis was done with the use of SPSS version 16. Level of significance was set at a P value of  $< 0.05$ .

**Results:** Of the 356 participants studied, 216 (60.7%) were non-reactive and 140 (39.3%) were reactive with the use of rapid screening methods. Whereas, with the use of ELISA screening method, 178 (50.0%) of the same recruited population tested reactive and the remaining 178 (50.0%) were non-reactive. In this study, rapid screening methods for HIV antibodies was found to be less sensitive compared with the use of ELISA for HIV P24 core antigen,  $p$  value = 0.001. The true sensitivity of rapid screening method when compared with ELISA method was found to be 69.7% but with 91.0% specificity. The positive predictive value was 88.6% and a negative predictive value of 75.0%.

**Conclusions:** Identifying new interventions for the prevention of HIV infection must remain a research priority. Most rapid screening methods still have sensitivity and specificity below the WHO recommendation. Until rapid screening improves, ELISA for P24 core antigen will be a preferred screening method for the high risk group. In the alternative, combination of two rapid screening with at least one having a specificity of 100% and the other having a sensitivity of 100% should be used to meet WHO recommendations.

**KEYWORDS:** ELISA, HIV High Risk Group, HIV Screening, PLWHA

### INTRODUCTION

HIV infection started as a disease of homosexuals in 1981 but now with a rapid heterosexual spread.<sup>1</sup> It is a retroviral infection that can be contacted vertically or horizontally via contact with infected body fluids, blood and semen especially. It is currently the fourth leading cause of mortality in the world, with a worldwide HIV prevalence of

33.4 million and annual incidence rate of 2.7million.<sup>1</sup> Two-third of the PLWHA is located in the sub-Saharan Africa.<sup>1</sup> In the literature, HIV counselling, testing and use of condom has been found to result in reduction in prevalence of HIV infection.<sup>1-3</sup>

New strategies to eradicate this global HIV/AIDS epidemic or at least prevent further transmissions are explored day-in day-out by researchers. Testing for HIV is a very important component of these HIV/AIDS prevention strategies due to the fact that an alarming number of the PLWHA remain unaware of their infection. Hence, they spread the infection easily in the community, especially, the high risk group. The high risk group are the people whose probability of HIV infection is above the average for the population in question. Examples are: sex partners of PLWHA, homosexuals, children born to HIV reactive mothers and clients with multiple sex partners.

Despite infection of about 50 million individuals with HIV, not up to 1,000 cases have been diagnosed in the first month of infection.<sup>3</sup> This is primarily because of lack of specific and recognisable acute retroviral syndrome.<sup>3</sup> It is also because, the generally available and affordable screening methods involve detection of viral antibodies which takes 26-35 days following initial infection to build up.<sup>3</sup> On the other hand, viraemia is detectable prior to symptoms in the form of HIV P24 antigen {using ELISA or HIV RNA/nucleic acid amplification}, usually between 9-11 days following initial infection.<sup>3</sup> More still, secondary transmission from an acutely infected index case to a susceptible sero-discordant partner has been documented to begin as early as 7-14 days after initial infection.<sup>3</sup> In 75% of persons, antibodies are produced in 4 to 8 weeks. In almost all persons, antibodies are produced within 14 weeks.

Traditionally, HIV testing is done with Western blot. However, with the advent of rapid HIV screening methods, HIV screening process has been transformed; enabling low resource countries to test more people without the use of laboratory or expensive equipment. The rapid screening test strips can be stored at room temperature contrary to what obtains with conjugates used in ELISA test. The results are ready within 20-30 minutes and easy to read, unlike what obtains with the traditional methods. In United States for instance, as high as 25% of those screened with ELISA did not return for their results.<sup>4</sup> Likewise, Chen et al found 64% of their respondents indicating a preference for rapid HIV testing and 74% indicated that if rapid oral HIV testing was available at a clinic they would test for HIV more frequently.<sup>5</sup>

However, rapid HIV testing is not without drawbacks. It has missed 3-4% of the ELISA detectable HIV infections.<sup>3,6</sup> High rates of false positives and false negatives from non-uniform staff training standards and what test strips were quoted with by the manufacturer are potential problems.<sup>7</sup> From the literature, most rapid tests have sensitivity and specificity below WHO recommendation of at least specificity of 98% and sensitivity of 99%.<sup>8</sup> Meanwhile, such tests like ELISA and nucleic acid amplification remain relatively expensive and have not been routinely used for clinical HIV screening. The aim of the study was to determine significant difference, if any, in the prevalence of HIV infection among high risk group if tested with more than one method of screening for HIV infection.

## **MATERIALS AND METHODS**

This study was carried out at LAUTECH Teaching Hospital, Osogbo. The Hospital serves as a referral Centre to care for PLWHA. Sample size was determined to be 356 patients, using Fisher's formula, with HIV sero-discordance prevalence set at 52%<sup>2</sup> and precision of 95% confidence interval. Ethical clearance was obtained from the ethics committee of LAUTECH Teaching Hospital as well as informed consent from patients studied with right to opt-out anytime in the course of the study without suffering any discrimination. Data obtained were confidentially kept.

**Sampling Procedure/Technique:** PLWHA were randomly selected during a support group meeting that usually bring all our HIV clients together. Their sex partners were traced, interviewed and tested. Exclusion criteria included non-consented partner or those that could not be reached.

**Data Collection:** All clients that met the inclusion criteria were made to undergo counselling and confidential testing for HIV infection following an informed consent. Testing was done with the use of rapid tests (parallel use of determine strips and Uni gold kits with Stat pack as a tie breaker), as well as with a fourth generation ELIZA kit to detect P24 HIV core antigen in the sera from 2.5mls of whole blood which was aseptically collected by veno-puncture.

**Rapid Screening Method:** Universal safety precaution and package insert instructions were ensured when handling specimens and work areas were kept clean and organized. Test procedure entailed labelling each package with patient's identification number. Fifty micro-litre of blood sample was aseptically taken with a pipette into Well "A" of the Uni gold®/Stat pack cassette/determine strip. Two drops of buffer was applied to the sample pad. A period of 10-20 minutes was allowed before the test result was read. The test was reactive i.e. positive if there were two distinct red lines in both the "control" and "test" regions. The test was nonreactive i.e. negative, if one red line appears in the "control" region and no line in the "test" region. Post-test counselling was done as soon as feasible and necessary instruction/treatment given.

**ELISA Screening Method:** The test reagent used was a fourth generation ELIZA kit (Gen screen ULTRA HIV Ag-Ab) produced by BIORAD®. The kit tests concurrently for HIV 1 and 2 P24 antigen (positive and negative control are included alongside the test sample). Test procedure entails removal of the Biorad® devices from package, and labelling it with client identification number. Sera was separated and incubated in the kit well at 37°C in the presence of 25 micro-litre of conjugate 1 for one hour.

The wells were then serially washed five times at 30 seconds soak interval using a washer which uses wash solution (0.37ml) during each washing cycle. The plates were further exposed to 100 micro-litre of conjugate 2 at 25°C for 30 minutes, after which a repeat of 5 washing cycle is carried out. Substrate solutions (70 micro litre of chromogen) were subsequently added and incubated inside the dark box for another 30 minutes. Once brought out of the dark box, 100 micro litre of a stop solution is added to stop all on-going reactions. Consecutively, Optical Density of endpoints was read at 450nm wavelength without differential filter, after stopping the solution.

Interpretation of results was determined by cut-off points. Post-test counselling with necessary treatment was also carried out based on the result of the test.

**Data Analysis:** Data analysis was done with the use of SPSS version 16. Level of significance was set at a P value of < 0.05.

## RESULTS

Of the 356 tested clients, there were 166 (46.7%) males and 190 (53.3%) females. Of these females, 136 (71.6%) were non-pregnant women while 54 (28.4%) were pregnant. A total of 178 (50.0%) tested subjects were reactive and the remaining 178 (50.0%) tested non-re active with the use of ELISA screening, Table 1. Thirty-four (63.0%) of the pregnant women tested reactive and 20 (37.0%) were non-reactive while, in women who were not pregnant, 62 (45.6%) was HIV reactive and 74 (54.4%) being HIV non-reactive (p value of 0.020), table 2. On the other hand, with use of rapid screening

methods, only 140 (39.3%) were reactive, Table 1. Twenty-four (44.4%) of the pregnant women tested reactive and 30 (55.6%) were non-reactive while, in women who were not pregnant, 58 (42.6%) was HIV reactive and 78 (57.4%) being HIV non-reactive (p value of 0.463) Table 3.

Comparison of rapid screening and ELISA on table 4, 124 (69.7%) of the tested clients were truly positive and 16 (9.0%) were false positives. Likewise, 162 (91.0%) were truly negative and 54 (30.3%) were false negatives. The true sensitivity of rapid screening method when compared with ELISA method was calculated to be 69.7% but with 91.0% specificity. The positive predictive value was 88.6% and a negative predictive value of 75.0%.

The true sensitivity of rapid screening method when compared with ELISA method was found to be 70.6% with 100% specificity in pregnant women. The positive predictive value was also 100% and a negative predictive value of 66.7%. Among the non-pregnant women on the other hand, the true sensitivity of rapid screening method when compared with ELISA method was 75.8% and 85.1% specificity. The positive predictive value was 81.0% with a negative predictive value of 80.8% table 5.

### Table of Results

**Table 1: Human Immunodeficiency Virus Status of Tested Clients with the Use of Rapid Screening and ELISA Methods (n = 356)**

HIV Status	Rapid Test Methods (%)			ELISA for P24 Core Antigen (%)		Total (%)
	Reactive	Non-Reactive	Total	Reactive	Non-Reactive	
Male clients	56 (33.7)	110 (66.3)	166 (100)	80 (48.2)	86 (51.8)	166 (46.6)
Female clients:-	84 (44.2)	106 (55.8)	190 (100)	98 (51.6)	92 (48.4)	190 (53.4)
(a) Pregnant	24 (44.4)	30 (55.6)	54 (100)	34 (63.0)	20 (37.0)	356 (100)
(b) Non-Pregnant	58 (42.6)	78 (57.4)	136 (100)	62 (45.6)	74 (54.4)	54 (28.4)
						136 (71.6)
<b>Total</b>	<b>140 (39.3)</b>	<b>216 (60.7)</b>	<b>356 (100)</b>	<b>178 (50.0)</b>	<b>178 (50.0)</b>	<b>356 (100)</b>

**Table 2: Comparison of Pregnancy Status and HIV Status Using ELISA (n=190)**

Factors	Respondents HIV Status with ELISA Screening			
	Reactive	Non-Reactive	Total	P Value
Pregnant	34 (63.0)	20 (37.0)	54 (100)	
Not pregnant	62 (45.6)	74 (54.4)	136 (100)	
<b>Total</b>	<b>96 (50.5)</b>	<b>94 (49.5)</b>	<b>190 (100)</b>	<b>0.020*</b>

Statistically significant

**Table 3: Comparison of Pregnancy Status and HIV Status Using Rapid Screening Methods (n=190)**

Factors	Clients HIV Status with Rapid Screening Methods (%)			
	Reactive	Non-Reactive	Total	P Value
Pregnant	24 (44.4)	30 (55.6)	54 (100)	
Not pregnant	58 (42.6)	78 (57.4)	136 (100)	
<b>Total</b>	<b>82 (43.2)</b>	<b>108 (56.8)</b>	<b>190 (100)</b>	<b>0.463</b>

Not statistically significant

**Table 4: Comparison of HIV Statuses with Rapid Screening and ELISA Methods (n=356)**

HIV Status with Rapid Screening Methods	HIV Status with the Use of ELISA Method (%)			
	Reactive	Non-Reactive	Total	P-Value
Reactive	124 (69.7)	16 (9.0)	140 (39.3)	
Non-reactive	54 (30.3)	162 (91.0)	216 (60.7)	
<b>Total</b>	<b>178 (100)</b>	<b>178 (100)</b>	<b>356 (100)</b>	<b>0.001*</b>

Statistically significant

**Table 5: Comparison of HIV Statuses with Rapid Screening and ELISA Methods among Pregnant and Non-Pregnant Women (n= 54/136)**

HIV Status with Rapid Screening Methods	HIV Status with the Use of ELISA Method (%)			
	Reactive	Non-Reactive	Total	P-Value
<b>Pregnant Women</b>				
Reactive	24 (70.6)	-	24 (44.4)	0.02*
Non-reactive	10 (29.4)	20 (100)	30 (55.6)	
<b>Total</b>	<b>34 (100)</b>	<b>20 (100)</b>	<b>54 (100)</b>	
<b>Non-Pregnant Women</b>				
Reactive	47 (75.8)	11 (14.9)	58 (42.6)	
Non-reactive	15 (24.2)	63 (85.1)	78 (57.4)	
<b>Total</b>	<b>62 (100)</b>	<b>74 (100)</b>	<b>136 (100)</b>	

Statistically significant

## DISCUSSIONS

A significant difference was observed among the results obtained with the use of ELISA and rapid screening methods (p value 0.001). The sensitivity of rapid screening method when compared with ELISA method was 69.7%. However, it has a higher specificity. This implies that when rapid screening test is reactive, patient is likely to be truly positive but if non-reactive, further methods of screening might be of help in this high risk group for HIV infection. Such disparity in results (false negative) had earlier been reported in the literature as possibly due to incubation period, level of sensitivity of method used, wrong technique, expired or substandard kits.<sup>6,7,10</sup> In this study, window period of HIV infection and level of sensitivity of method used may likely account for the disparity in results.

Moreover, it takes about 26-35 days following initial HIV infection before viral antibodies are detected by rapid screening method whereas ELISA for P24 core antigen might detect HIV infection as early as second week (9-11 days) of infection.<sup>3</sup> In both low and high prevalence areas, HIV antibody tests have missed 3-4% of HIV infection that was detectable by the use of antigen tests.<sup>3,6</sup> Meanwhile, acutely infected PLWHA can infect susceptible acquaintances as early as 7-14 days following initial infection.<sup>3</sup>

In addition, HIV variants differ in nucleotide sequence and shows varied geographical and epidemiological distribution.<sup>11</sup> Meanwhile, recombinant protein from a particular prototype virus alone might be used to prepare the rapid screening kit, hence the difference in specificity and sensitivity of the same kit at different regions.<sup>11</sup> Most rapid test kits for HIV utilize only the g41 antigen as the target antigen.<sup>8</sup> Therefore, failure of Rapid screening test to detect HIV reactive samples may be due to inadequate coating of the antigens, nature of the antigen used and genetic heterogeneity of the virus.<sup>8,11</sup> A sensitivity of 69.7% and specificity of 91.0% of this study is below WHO recommendation of at least 99% and 98% for sensitivity and specificity of rapid screening methods respectively.<sup>8</sup> In Cameroon, determine strip had a high sensitivity of 100% but specificity of 91.5% similar to what obtained in this study.<sup>9</sup> The study however recorded a high false positive result, similar to 10.5% false positive result obtained in Eastern Democratic Republic of Congo.<sup>8</sup>

The positive predictive value of 88.6% and a negative predictive value of 75.0% of this study implies that 11.4% of those truly HIV positive and 25% of those truly HIV negative were missed. When extrapolated to the universe, hundreds of thousands of mis-diagnoses results. False negative results are a threat to both the public and individual health prevention strategies. Such individual may not seek other testing opportunities and continue to take HIV preventive measures rather than receiving the care and treatment needed, thereby infecting others unknowingly. Likewise, a false positive result makes the client to undergo unnecessary psychological trauma and care.

Ideally, testing sensitivity, specificity and positive predictive values for a single test should all meet WHO standards.<sup>8</sup> However, until rapid screening improves, a combination of two rapid tests with one having sensitivity close to 100% and the other having a specificity near 100% is advisable. When the above rule was applied in a previous study, testing sensitivity increased from 68.7% to 93.5%; an additional improvement to 95.1% was obtained after tightening quality control measures and re-training of staff. Similarly in a study conducted in Cameroon, specificity of Determine strip was 91.5%. However, when combined with another rapid screening method (Immunocombii), sensitivity and specificity increased to 100% and 98.8%<sup>10</sup> respectively. It thus satisfied the WHO recommendation and with much reduced false positives. HIV rapid screening tests should be validated locally and made to undergo external quality assurance prior to use.<sup>4</sup> Everett in a sensitivity and specificity study of Capillus and Determine strips, respectively found 98.6% and 99.7% in Capillus and 95.1% & 99.7% for Determine strips while a parallel use both strips revealed a sensitivity of 98.6% and 100% specificity.<sup>4</sup>

At present, Western Blot and HIV nucleic acid amplification are the conventional screening methods and not yet widespread in Nigeria. Besides, it is still very expensive, thus, the need to employ use of other methods. Chen et al found homosexuals to prefer rapid HIV testing more than ELISA screening.<sup>5</sup> It therefore promotes uptake of VCT for HIV.

However, when rapid screening was compared with ELISA screening technique, Torane et al has recommended the later as the preferred option for screening of healthy blood donors,<sup>11</sup> similar to Mabayoje's findings.<sup>6</sup> Similarly, partners of PLWHA should be screened with more sensitive and specific HIV tests than rapid screening methods to ascertain their HIV statuses. This is because they belong to the high risk group for acquisition of HIV infection. However, with the emerging concept of specimen pooling for HIV RNA testing/ELISA can make them to become both clinically and economically feasible.<sup>3,12</sup> This concept involves pooling aliquots of 200microlitre of sera from 90 HIV antibody-negative patients as a single pool. Testing individual specimen is only required if there is a positive result in the master pool. This pooling was also found to be advantageous in reducing the false positive result which can be as high as 1% for nucleic acid amplification test for HIV.<sup>3,12</sup>

Among the female population in this study, pregnancy status was found to be statistically significant in acquisition of HIV infection (p value of 0.020) when status was determined with the use of ELISA test for P24 HIV core antigens. However, this significance was lacking with the use of rapid screening test for HIV antibody (p value = 0.463). Likewise, this study shows a lower true sensitivity of rapid screening method in pregnant women when compared with non-pregnant women (70.1 versus 75.8%) but its positive predictive value is 100% in them. Pregnancy has been reported to induce an altered immune state to protect the fetus from immune rejection.<sup>13,14</sup> Similarly, a study from Rakai in Uganda reported that the risk of HIV acquisition rises during pregnancy with an incidence rates of 2.3/100 person years, compared to 1.1/100 person years in non-pregnant and non-lactating woman and 1.3/100 person years during lactation.<sup>13</sup> This was explained as possibly due to hormonal changes affecting the genital tract mucosa or immune responses.<sup>15</sup>

## CONCLUSIONS

Identifying new interventions for the prevention of HIV infection must remain a research priority. Most rapid screening methods still have sensitivity and specificity below WHO recommendation. Until rapid screening improves, ELISA for P24 core antigen is a preferred screening method for people in the high risk group of contracting HIV infection. In the alternative, combination of two rapid screening with at least one having a specificity of 100% and the other having a sensitivity of 100% should be used.

In this study, the use of ELISA kit to detect HIV P 24 core antigens resulted in more of the tested clients being found to be sero-positive, especially among the pregnant population (immune-suppressed). However, it has been difficult to determine whether there was a high false positive result with the use of ELISA for HIV P24 core antigen or if there was high false negative result with the use of rapid tests for HIV antibody. A further study with the test for HIV DNA/RNA amplification being incorporated as controls is therefore recommended.

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